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THE EFFECT OF SALINITY ON THE AMINO ACID CONCENTRATION IN RANGIA CUNEATA (PELECYPODA)

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Simpson *et al.* (1959) reported that the amino acid taurine was in high concentration in marine molluscs. Taurine could not be detected by means of paper chromatography in extracts of fresh-water and terrestrial molluscs. Allen and Awapara (1960) studied the metabolism of S-35 methionine in the mussel, *Mytilus edulis,* and the brackish-water clam, *Rangia cuneata.* Taurine was not detectable in the tissues of the latter. Following injections of radioactive methionine, both species were found capable of degrading methionine to taurine and sulfate. In *R. cuneata* taurine was formed but was rapidly metabolized and excreted as an unknown compound. Taurine was retained in high concentration in the tissues of *M. edulis.*

These results suggested that the high concentration of taurine and perhaps other amino acids may be associated in some way with the salinity of the environment. To investigate this possible relationship, *R. cuneata* was gradually moved from water of 3‰ to water of 25‰. Amino acid extracts were made from specimens at several salinities within the above range, and four individual amino acids were measured. The results of this study showed that taurine was not retained in the tissues of *R. cuneata* regardless of salinity, but that the amino acids, particularly alanine, increased with the higher saline environments.

Materials and Methods

R. cuneata was collected in the San Jacinto River, Houston, Texas. Following collection, the clams were transported to the laboratory and kept at approximately 25° C. in large aerated 50-gallon aquaria. The clams were allowed at least three days to adapt to the aquarium before being used in experimental work.

A series of five-gallon aquaria were set up in consecutive order of increasing salinities which ranged from 3‰ to 25‰. Filtered sea water was diluted to the appropriate volume with distilled water. Salinities were determined by the Mohr

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technique, as described in Standard Methods (1955). Several tests were made on *R. cuneata* to determine how rapidly they could be moved from one salinity to another. It was found that if the animals were allowed to remain in a particular tank for two days, they could be transferred without fatalities.

In order to insure correct controls for starvation a group of clams was started together in the fresh-water tank. After two days all but a dozen of the original group were moved to the next tank of higher salinity. This was repeated until all the tanks contained a dozen clams. After the final tank was reached, two days were allowed to elapse prior to making amino acid extracts from representatives of each tank. The effects of leaving clams in one tank over a period of time were checked, and up to 20 days there was no measurable change in the amino acid concentration.

The extraction and fractionation of amino acids were carried out as reported by Allen and Awapara (1960). The amino acids were analyzed by two-dimensional ascending paper partition chromatography. The amino acid extracts were spotted on Whatman filter paper #4. Solvent mixtures were 72% phenol and 65% lutidine. Both solvents were redistilled prior to use.

The quantitative estimation of alanine, aspartic acid, glutamic acid and glycine was carried out by the method of Awapara *et al.* (1950) with the following modification from Moore and Stein (1948). After localizing the amino acids on the chromatogram the spots were outlined with pencil and the paper sprayed with 1% KOH in methanol. After drying the sprayed papers in air, the spots were cut out and placed in test tubes. The tubes were put in a vacuum desiccator over concentrated sulfuric acid for three hours. Color was developed with the reagent of Moore and Stein (1948). The tubes were read at 570 mμ on a Coleman Junior Spectrophotometer.

The dry weight was determined by drying the total animal minus shell at 100° C. until the weight remained constant. The inorganic constituents (ash weight) were estimated by incinerating the dry tissues in a muffle furnace at 1000° C. The difference in dry and ash weights was expressed as per cent ash weight.

Results and Discussion

In view of the possible relationship between amino acid concentration and the hydration of tissues, the dry weight and ash weights were determined on specimens of *R. cuneata* from environments of different salinities. The results are shown in Table I.

From the dry weight studies, it is obvious that as the clams progress from a dilute to a more saline environment there is a loss of water. The ash weights, on the other hand, increase slightly in the higher salinities, reflecting an increase of inorganic constituents. These results are in agreement with earlier investigations of Fredericq (1904), Krogh (1939), and Fox (1941). These investigators demonstrated that euryhaline molluscs establish an equilibrium with their environment with respect to inorganic ions. This equilibrium is observed by a weight-volume change and a fluctuation of ion concentration depending on the salinity of the environment.

The effect of increased salinity on the amino acid concentration in *R. cuneata* is shown in Table II and Figure 1. All four amino acids increased in concentration

until the animals reached a salinity of 17 ‰. In the two higher salinities the amino acids decreased in concentration. From the data on per cent dry weights and ash weights it appears as if the loss of tissue water and gain of inorganic ions reaches a maximum in salinities of 17–20‰. This leveling off of water loss and the decrease in inorganic ion exchange in *R. cuneata* suggests a shift in osmotic control. The concurrent decrease in amino acid concentration may be associated with this phenomenon. This possible relationship between amino acids, the control of tissue water, and inorganic ions needs further investigation. These three factors may prove to be in dynamic equilibrium in euryhaline molluscs when these animals are

TABLE I

Per cent dry weights and ash weights of R. cuneata *from different salinities*

Salinity		Per cent dry wgts.	Per cent* ash wgts.
3‰	Range	18.18–20.38	2.56–4.54
	Mean	19.34	3.43
	S.D.	0.36	0.36
6‰	Range	19.21–21.40	3.54–4.43
	Mean	20.36	3.95
	S.D.	0.41	0.13
10‰	Range	21.06–24.00	3.75–4 97
	Mean	23.18	4.41
	S.D.	0.51	0.24
17‰	Range	23.54–25.76	4.31–5.97
	Mean	24.41	5.13
	S.D.	0.95	0.35
20‰	Range	25.03–26.82	5.14–6.31
	Mean	25.75	5.84
	S.D.	0.33	0.22
25‰	Range	24.62–26.86	4.63–6.12
	Mean	25.70	5.27
	S.D.	0.32	0.23

* Per cent ash weight expressed in terms of dry weight of animal.

moved from one environment to another. If such is the case, the inclusion of amino acids in osmotic control broadens considerably ideas encompassed in the process of osmoregulation.

Whether or not this increase in alanine is in response to an osmotic imbalance cannot be determined from these results. However, some studies have shown indirectly that amino acids play a role in osmoregulation. Camien *et al.* (1951) and Duchâteau *et al.* (1952) have shown that marine invertebrates have a higher concentration of free amino acids than fresh-water forms. This fact was the basis for investigating the effect of salinity change on euryhaline crabs by Duchâteau and Florkin (1955) and Shaw (1958a, 1958b, 1959). These investigations showed

that the amino acid concentration fluctuated in direct proportion with the salinity of the environment. Likewise, Duchâteau *et al.* (1961) moved the polychaet worm, *Arenicola marina,* from 100% sea water to 50% sea water, and observed a decrease in the amino acid concentration. The amino acids most affected by the change were glycine and alanine. The total decrease in amino acids was more than what would be accounted for on the basis of tissue hydration. Potts (1958) reported the effect of changing salinity on two molluscs, *Mytilus edulis* (marine) and *Anodonta cygnea* (fresh-water). The amino nitrogen decreased in *M. edulis* as this animal was moved into water of lower salinity. The reverse was true when

TABLE II

Amino acid concentration in R. cuneata *from different salinities (expressed as μmoles/gm. tissue dry weight)*

Salinity		Alanine	Glycine	Glutamic	Aspartic
3‰	Range*	3.2–9.6	3.0–6.7	3.2–6.2	1.1–3.4
	Mean	6.1	5.1	4.3	2.1
	S.D.	1.1	0.7	0.4	0.4
6‰	Range	14.3–24.3	14.5–26.4	10.1–14.7	2.3–6.0
	Mean	18.4	18.9	13.3	3.5
	S.D.	1.9	2.1	0.8	1.1
10‰	Range	82.9–108.6	29.0–38.4	19.9–22.8	5.7–9.5
	Mean	92.6	34.8	21.4	7.7
	S.D.	4.3	1.7	0.6	0.7
17‰	Range	205.7–249.2	45.9–58.2	29.5–35.8	14.5–17.4
	Mean	225.4	51.6	32.3	16.2
	S.D.	7.6	2.4	1.3	0.5
20‰	Range	179.5–209.3	26.6–36.0	21.0–29.2	8.9–12.8
	Mean	192.6	32.1	24.4	10.3
	S.D.	5.1	1.5	1.6	0.8
25‰	Range	156.5–186.7	20.8–25.6	18.3–21.6	5.0–9.0
	Mean	170.2	23.7	20.0	6.9
	S.D.	5.1	0.8	0.6	0.8

* Range represents distribution of amino acid concentrations from five separate individual animals from each salinity.

A. cygnea went into higher salinities. There is an effect of salinity on the amino acid concentration in euryhaline invertebrates. The exact relationship between amino acids, water balance and inorganic constituents needs to be worked out.

The source of alanine as well as the other measured amino acids cannot be explained readily. They may arise from the increased hydrolysis of protein. However, the high concentration of one amino acid such as alanine suggests that there may be more involved than just increased protein breakdown. A process whereby one amino acid could reach such high values could be explained by the interaction of the amino acids from protein and keto-acids from carbohydrate

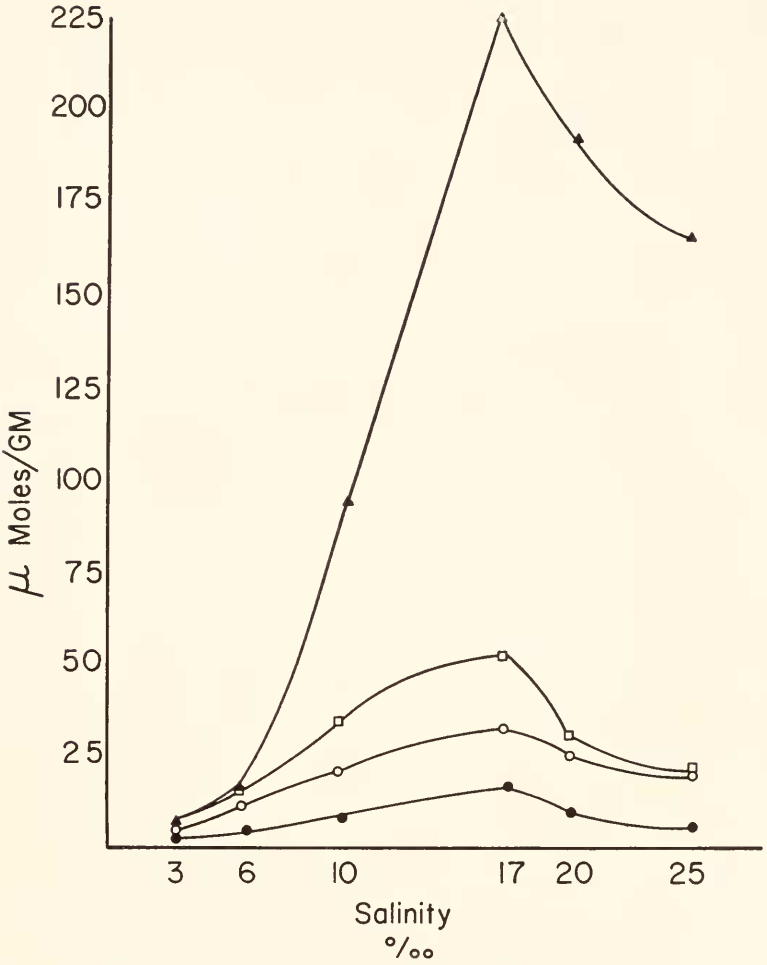

FIGURE 1. Amino acid concentration in *Rangia cuneata* taken from different salinities. Alanine ▲; aspartic acid ●; glutamic acid ○; glycine □.

metabolism via transamination. There is little evidence for this. If such were the case one might expect to observe an increase of respiration, a decrease in glycogen and an increase in one or more amino acids. Pieh (1936) and Maloeuf (1938) observed an increase in the respiration of *M. edulis* as this mussel was placed in dilute sea water. This was also true when the animals were returned to 100% sea water. Preliminary studies in this laboratory have shown a decrease in glycogen as *R. cuneata* is moved into higher salinities. These results may be interpreted as an indication of work being done in order to maintain osmotic equilibrium. At the same time carbohydrate is being used for energy purposes, the production of keto-acids provides a source of carbon chains for the formation of amino acids via transamination. Only experimental evidence will verify the latter.

Summary

1. The amino acids alanine, glycine, glutamic acid and aspartic acid were quantitated in *Rangia cuneata* taken from different salinities.

2. The individual amino acids increased in concentration as the salinity increased.

3. The increased concentration of individual amino acids followed a definite pattern which was already established prior to the change into higher salinities. The pattern was: alanine > glycine > glutamic acid > aspartic acid, regardless of environment.

4. The possible role of amino acids in an animal withstanding changes in salinity is discussed.

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